UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

December 14, 2005

MEMORANDUM

Subject: Efficacy Review for Aseptrol S10-Tab, EPA Reg. No. 70060-19; DP Barcode: D321735

From: Ibrahim Laniyan, Microbiologist
Product Science Branch
Antimicrobials Division (7510C)

Thru: Nancy Whyte, Acting Team Leader
Product Science Branch
Antimicrobials Division (7510C)

December 14, 2005

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

To: Wanda Henson / Emily Mitchell
Regulatory Management Branch II
Antimicrobials Division (7510C)

Applicant: Engelhard Corporation
101 Wood Avenue
Iselin, NJ 08830-0770

Formulation from the Label:

Active Ingredient(s)	% by wt
Sodium Chlorite	20.8 %
Sodium Dichloroisocyanurate dihydrate.	7.0 %
Other Ingredients	72.2 %
Total	100.0 %

I. BACKGROUND

The product, Aseptrol S10-Tab (EPA Reg. No. 70060-19), is an EPA-approved disinfectant (bactericide, tuberculocide, virucide) for use on hard, non-porous surfaces in industrial, institutional, animal care, and hospital or medical environments. The applicant requested that EPA amend the product's registration to include a claim for effectiveness as a sterilant. The applicant also requested that the product's registration be amended to include claims for use of the product as a disinfectant against additional microorganisms (i.e., Foot and Mouth Disease virus, Hepatitis B virus, Hepatitis C virus, Newcastle disease virus, Norwalk virus, *Escherichia coli* O157:H7, *Staphylococcus aureus* - MRSA, and *Enterococcus faecalis* VRE). Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; and MicroBioTest, Inc., located at 105B Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant's representative to EPA (dated September 6, 2005), nine studies (MRID Nos. 466417-01 through 466417-09), Statements of No Data Confidentiality Claims for all nine studies, and the proposed label.

II. USE DIRECTIONS

The product is designed for sterilizing surfaces, such as manufacturing and laboratory equipment. Directions on the proposed label provided the following information regarding preparation and use of the product as a sterilant: Prepare a 1000ppm use solution by adding eight 6.0-gram tablets of the product to 1 gallon of water. Apply the use solution by either thoroughly soaking or wetting the target surface or by immersion. Treated surfaces must remain wet for at least 1 hour. Allow surfaces to air dry. [Note: The directions did not specify a pre-cleaning step. The directions did not specify the length of time that the tablets should be left to react with the diluent (i.e., to activate the product.)]

The product is also designed for disinfecting pre-cleaned, hard, non-porous surfaces, such as bio-safety hoods, cages, coops, counters, crates, floors, instruments, kennels, sinks, tiles, utensils, and walls. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant:

> As a disinfectant against bacteria: Pre-clean surfaces. Prepare a 200ppm use solution by adding two 6.0-gram tablets of the product to 1 gallon of water. Wait 15 minutes for the tablets to completely dissolve. Apply the use solution thoroughly wetting surfaces with a cloth, mop, sponge, or sprayer, or by immersion. Treated surfaces must remain wet for 10 minutes. Wipe dry with a cloth, sponge, or mop, or allow to air dry.
>
> As a virucide (against Hepatitis B virus, Hepatitis C virus, New Castle Disease virus, Norwalk virus, Human coronavirus): Pre-clean surfaces. Prepare a 200ppm use solution by adding two 6.0-gram tablets of the product to 1 gallon of water. Wait 15 minutes for the tablets to completely dissolve. Apply the use solution using a cloth, mop, sponge, or sprayer, or by immersion. Treated surfaces must remain wet for 10 minutes. Wipe dry with a cloth, sponge, mop or allow to air dry.
>
> As a virucide against the Foot and Mouth Disease virus: Pre-clean surfaces. Prepare a 100ppm use solution by adding one 6.0-gram tablet of the product to 1 gallon of water.

Wait 15 minutes for the tablet to completely dissolve. Apply the use solution using a cloth, mop, sponge, or sprayer, or by immersion. Treated surfaces must remain wet for 30 minutes. Wipe dry with a cloth, sponge, mop or allow to air dry.

The product is also designed for sanitizing pre-cleaned, hard, non-porous, food-contact surfaces, such as utensils. Directions on the proposed label provided the following information regarding preparation and use of the product as a sanitizing rinse: Remove gross food particles and soil by a pre-flush, pre-scrape, or pre-soak. Thoroughly wash surfaces with a good detergent. Rinse with potable water. Prepare a 50ppm use solution by adding two 1.5-gram tablets of the product to 2 quarts of water. Wait 10 minutes for the tablets to completely dissolve. Apply the use solution using a cloth, mop, sponge, coarse sprayer, or by immersion. Treated surfaces must remain wet for at least 60 seconds. Allow surfaces to drain and air dry. Do not rinse.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sterilizers: The AOAC Sporicidal Test is required for substantiating sterilizing claims. The following information applies to all products represented as sporicidal or sterilizing agents. Sixty carriers, representing each of 2 types of surfaces (porcelain penicylinders and silk suture loops), must be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 product samples representing 3 different batches, one of which is at least 60 days old (240 carriers per sample; a total of 720 carriers). Any sterilizing agent (liquid, vapor, or gas) that is recommended for use in a specific device must be tested by the AOAC Sporicidal Test in that specific device and according to the directions for use. **Performance Standard:** Killing on all of the 720 carriers is required; no failures are permitted. Data to support sterilizing claims must be confirmed by tests conducted by a second, independent laboratory of the applicant's choice (other than the laboratory that developed the original data). The following minimal confirmatory data must be developed on one sample of the product: Thirty carriers with each of the 2 types of surfaces (silk suture loops and porcelain penicylinders) against spores of both *Bacillus subtilis* and *Clostridium sporogenes* (a total of 120 carriers) by the AOAC Sporicidal Test. These Agency standards are presented in DIS/TSS-9.

Disinfectants for Use in Hospital or Medical Environments – Additional Microorganisms (Bactericidal requirements): Effectiveness of disinfectants against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, but not including viruses, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 samples, representing 2 different batches. **Performance requirements:** To support products labeled as "disinfectants" for specific microorganisms (other than those microorganisms named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data, on appropriate culture media, must be submitted on each test microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are presented in DIS/TSS-1.

Virucidal requirements: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of the

AOAC Use-Dilution Method (for liquid disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. **Performance standard:** For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides - Use of a Surrogate Virus: For certain viruses, there are no *in vitro* systems or *in vivo* animal models (except for humans and chimpanzees). The Agency permits the testing of surrogate viruses in these cases, for example, Bovine viral diarrhea virus as a surrogate for human Hepatitis C virus, Duck Hepatitis B virus as a surrogate for Human Hepatitis B virus, and Feline calicivirus as a surrogate for Norwalk virus. When a surrogate virus is used, confirmatory data must be developed by an independent laboratory on at least 1 product lot.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 466417-01 "Sporicidal Activity of Disinfectants" for Aseptrol S-Tab 10, by Amy S. Jeske. Study conducted at ATS Labs. Study completion date – May 6, 2005. Project Number A02698.

Note: The applicant provided this confirmatory study to validate an initial study (assigned MRID No. 459160-01), which was previously submitted to the Agency.

This study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). One lot (Lot No. 0407 22) of the product, Aseptrol S-Tab 10, was tested using the AOAC Sporicidal Activity of Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Testing was conducted on February 23, 2005 and March 24, 2005. A 1073ppm use solution (for studies conducted on February 23, 2005) and a 1084ppm use solution (for studies conducted on March 24, 2005) were prepared by adding eight 1.5-gram tablets of the product to a flask containing 1 L of 400ppm AOAC synthetic hard water (titrated at 402 and 404ppm). The flask was covered and allowed to stand closed for 30 minutes in the dark, and was then gently swirled to dissolve any undissolved solids. Thirty (30) porcelain penicylinder carriers and Dacron suture loops were contaminated with the test organism by immersing them for 15 minutes in a 72±4 hour old culture, at a ratio of 1 carrier per 1.0 ml of broth culture. The carriers were then dried in a vacuum dessicator for 2-3 days prior to use. Thirty (30) contaminated carriers were exposed in groups of five to 10ml of the use solution for a contact time of 1 hour at 18.0-19.0°C. After the contact time, each carrier was transferred to 10ml of Fluid Thioglycollate Medium with 0.3% sodium thiosulfate. Each carrier was then transferred to a second tube of Fluid Thioglycollate Medium. Subculture tubes were incubated for 21 days at 35-37°C. Following incubation, the subculture tubes were visually examined for growth. Tubes demonstrating no-growth were heat-shocked for 20 minutes at 80°C and re-incubated for 72±4 hours at 35-37°C. The heat-shocked tubes were stored at 2-8°C for 1 day

prior to examination. Controls included those for purity, sterility, viability, carrier quantitation, neutralization confirmation, and acid resistance at 2, 5, 10, and 20 minutes. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed using a Hach Digital Titrator.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The applicant provided data for a failed trial set up on February 23, 2005 against *Bacillus subtilis*. In that trial, the carrier quantitation counts (~10^3) were below the acceptance criterion ($\geq$1.0 x 10^4 CFU/carrier) for both the suture loops and porcelain penicylinders tested against *Bacillus subtilis*. Thus, the test was invalid. These data were not used to evaluate efficacy of the test product. The study was repeated on March 24, 2005 against *Bacillus subtilis*. See Attachment I of the laboratory report.

2. MRID 466417-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate for Norwalk and Norovirus – Confirmatory Assay" for Aseptrol S-Tab 10, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 24, 2005. Project Number A02887.

Note: The applicant provided this confirmatory study to validate an initial study (assigned MRID No. 462262-06), which was previously submitted to the Agency.

This study, under the direction of Study Director Mary J. Miller, was conducted against Feline calicivirus (F-9 strain; ATCC VR-782) using Crandel Reese feline kidney cells (CRFK cells; ATCC CCL-94; propagated in-house) as the host system. The study protocol followed ATS Labs Protocol No. LEH02021505.FCAL.1 (copy not provided). One lot (Lot No. 0407 22) of the product, Aseptrol S-Tab 10, was tested. A 196ppm use solution was prepared by adding three 1.5-gram tablets of the product to a container containing ½ gallon of 400ppm AOAC synthetic hard water (titrated at 398ppm). The container was covered, allowed to stand in the dark for 15 minutes, and then swirled to mix the use solution and dissolve any undissolved solids. The stock virus cultures contained a 5% organic soil load (fetal bovine serum). Two glass carriers were tested for the single product lot against the target virus. Films of virus were made by spreading 0.2ml of stock virus on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 19.8°C in a relative humidity of 67-69%. For the single product lot, 2.0ml of the use solution was added to the virus films for 10 minutes at 19.8°C. After the contact period, the virus-disinfectant mixture was scraped from the surface of each dish with a cell scraper. Each sample was passed through a pre-spun Sephadex column using a syringe plunger. Ten-fold serial dilutions were prepared, using Minimum Essential Medium containing 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 1.0ml of each dilution. The subcultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and microscopically scored periodically for 7 days for specified cytopathic effects, cytotoxicity, and viability. The log_{10} reduction in infectivity was calculated using the Most Probable Number (MPN) method. Controls included those for input virus titer, dried virus titer, neutralization, cytotoxicity, and data consistency. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed using a calibrated buret.

3. MRID 466417-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Newcastle Disease Virus" for Aseptrol S-Tab 10, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – September 9, 2004. Project Number A02343.

This study was conducted against the Newcastle disease virus (ATCC VR-108; Strain B1, Hitchner, or Blacksburg), using chicken embryo fibroblast cells (CEF cells; originally obtained from Charles River SPAFAS; propagated in-house) as the host system. Two lots (Lot Nos. 19125-1-1 and 19125-1-2) of the product, Aseptrol S-Tab 10, were tested according to ATS Labs Protocol No. LEH02021304.NEW (copy not provided). A 188ppm use solution (for Lot No. 19125-1-1) and a 216ppm use solution (for Lot No. 19125-1-2) were prepared by adding three 1.5-gram tablets of the product to a container containing ½ gallon of 400ppm AOAC synthetic hard water (titrated at 398ppm). The container was covered, allowed to stand in the dark for 15 minutes, and then swirled to mix the use solution and dissolve any undissolved solids. No organic soil load was present in the virus stock, and none was added. Films of virus were prepared by spreading 0.2ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 20.1°C in a relative humidity of 42% for 20 minutes. For each lot of product, separate dried virus films were treated with 2.0ml of the use solution for 5 minutes at 20.0°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in minimal essential medium supplemented with 2% heat-inactivated fetal bovine serum, 10µg/ml gentamicin, 100units/ml penicillin, 2.5µg/ml amphotericin B, 2.0mM L-glutamine, and 5% tryptose phosphate broth. CEF cells in multi-well culture dishes were inoculated in quadruplicate with 0.1ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus count, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed using a calibrated buret.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

4. MRID 466417-04 "Initial Virucidal Effectiveness Test Using Duck Hepatitis B Virus" for Aseptrol S-Tab 10, by M. Khalid Ijaz. Study conducted at MicroBioTest, Inc. Study completion date – November 1, 2004. Laboratory Project Identification Number 414-140.

This study, under the direction of Study Director M. Khalid Ijaz, was conducted against the Duck Hepatitis B virus (DHBV; strain not specified; obtained from HepadnaVirus Testing) using primary duck hepatocytes (cultures prepared using ducklings from Metzer Farms) as the host system. The study protocol followed a MicroBioTest Protocol titled "Initial Virucidal Effectiveness Test using Duck Hepatitis B Virus," dated June 9, 2004 (copy provided). Two lots (Lot Nos. 19125-1-1 and 19125-1-2) of the product, Aseptrol S-Tab 10, were tested. A 200ppm use solution was prepared by adding two tablets of the product to a foil-covered flask containing 1200.0 ml of 400ppm AOAC synthetic hard water (titration results not provided). The use solution was allowed to react in the dark for 10 minutes. The flask was then swirled to dissolve any undissolved solids. The stock virus cultures contained at least a 5% organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 ml of stock virus on the bottoms of separate sterile, glass Petri dishes. The

virus films were dried for 30-60 minutes at room temperature. For each lot of product, 2.0 mL of the use solution was added to the virus films for 10 minutes at 20±2°C. After the contact period, 2.0 ml of fetal bovine serum containing 0.3% sodium thiosulfate was added to neutralize the sample. The neutralized mixture was scraped from the surface of each dish with a cell scraper. Each sample was loaded onto pre-spun Sephacryl columns and passed through the column. Ten-fold serial dilutions were prepared, using Liebovitz-15 complete tissue culture medium. Primary duck hepatocytes were inoculated in quadruplicate with an unspecified amount of each dilution. The inoculum was allowed to adsorb for 20-30 hours at 37±2°C in 5±1% CO_2. Post-adsorption, the cultures were aspirated, washed once with test medium, and then re-fed with 2.0 ml of test medium. The cultures were incubated at 37±2°C in 5±1% CO_2 for 7-14 days. The plates were assayed by an immunofluorescence assay. The $\log_{10}$ reduction in infectivity was determined using the Most Probable Number (MPN) method. Controls included those for cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency. The equivalent free chlorine dioxide concentration of the prepared use solution was confirmed by an unspecified titration method.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Section E of the "Miscellaneous Information" section of the laboratory report (see page 25) states to "See attachments." The laboratory report does not have any attachments.

5. MRID 466417-05 "Confirmatory Virucidal Effectiveness Test Using Duck Hepatitis B Virus" for Aseptrol S-Tab 10, by Zheng Chen. Study conducted at *MicroBioTest, Inc. Study completion date* – December 23, 2004. Laboratory Project Identification Number 414-142.

This study, under the direction of Study Director Zheng Chen, was conducted against the Duck Hepatitis B virus (DHBV; strain not specified; obtained from HepadnaVirus Testing) using primary duck hepatocytes (cultures prepared using ducklings from Metzer Farms) as the host system. The study protocol followed a MicroBioTest Protocol titled "Confirmatory Virucidal Effectiveness Test Using Duck Hepatitis B Virus," dated June 9, 2004 (copy provided). One lot (Lot No. 19125-1-2) of the product, Aseptrol S-Tab 10, was tested. A 209ppm use solution was prepared by adding two tablets of the product to a foil-covered flask containing 1200 ml of 400ppm AOAC synthetic hard water (titration results not provided). The use solution was allowed to react in the dark for 10 minutes, and was then swirled to dissolve any undissolved solids. The stock virus cultures contained at least a 5% organic soil load (serum not specified). Two glass carriers were tested against the target virus. Films of virus were made by spreading 0.2 ml of stock virus on the bottoms of separate sterile, glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. The use solution was sprayed onto the virus films from a distance of 6-8 inches until the surface was totally wet. The virus films were allowed to remain wet for 10 minutes at 22°C. After the contact period, 2.0 ml of fetal bovine serum containing 0.3% sodium thiosulfate was added to neutralize. The neutralized mixture was scraped from the surface of each dish with a cell scraper. Each sample was loaded onto and passed through pre-spun Sephacryl columns. Ten-fold serial dilutions were prepared, using Liebovitz-15 complete tissue culture medium. Primary duck hepatocytes were inoculated in quadruplicate with an unspecified amount of each dilution and allowed to adsorb for 20-30 hours at 37±2°C in 5±1% CO_2. Post-adsorption, the media was aspirated. The cultures were washed, re-fed with 2.0 ml of test medium, and then incubated at 37±2°C in 5±1% CO_2 for 7-14 days.

The plates were assayed by immunofluorescence assay. The log_{10} reduction in infectivity was determined using the Most Probable Number (MPN) method. Controls included those for cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed by titration.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

6. MRID 466417-06 "Initial Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for Human Hepatitis C Virus" for Aseptrol S-Tab 10, by M. Khalid Ijaz. Study conducted at MicroBioTest, Inc. Study completion date – October 31, 2004. Laboratory Project Identification Number 414-141.

This study, under the direction of Study Director M. Khalid Ijaz, was conducted against the Bovine viral diarrhea virus (strain not specified; originally obtained from American BioResearch Laboratories; propagated in-house) using Madin Darby bovine kidney cells (MDBK cells; ATCC CCL-22) as the host system. The study protocol followed a MicroBioTest Protocol titled "Initial Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for human Hepatitis C virus)," dated June 9, 2004 (copy provided). Two lots (Lot Nos. 19125-1-1 and 19125-1-2) of the product, Aseptrol S-Tab 10, were tested. A 200ppm use solution was prepared by adding two tablets of the product to a foil-covered flask containing 1200 ml of 400 ppm AOAC synthetic hard water (titration results not provided). The use solution was allowed to react in the dark for 10 minutes, and was then swirled to dissolve any undissolved solids. The viral stock contained at least a 5% organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 ml of stock virus on the bottoms of separate sterile, glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. The use solution was sprayed (4 spray pumps) onto the virus films from a distance of 7 inches until the surface was totally wet. The virus films were allowed to remain wet for 10 minutes at 20±2°C. After the contact period, 2.0 ml of horse serum containing 0.3% sodium thiosulfate was added to neutralize. The neutralized mixture was scraped from the surface of each dish with a cell scraper. Each sample was loaded onto pre-spun Sephacryl columns and spun to extract the eluate. Ten-fold serial dilutions were prepared, using Minimum Essential Medium Eagle's containing 5% horse serum. MDBK cells were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at 37±2°C in 5±1% CO_2 for 3-5 days. The plates were assayed by a direct immunofluorescence assay. The log_{10} reduction in infectivity was determined using the Most Probable Number (MPN) method. Controls included those for cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed by titration.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

7. MRID 466417-07 "Confirmatory Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for Human Hepatitis C Virus)" for Aseptrol S-Tab 10, by Zheng Chen. Study conducted at MicroBioTest, Inc. Study completion date – November 2, 2004. Laboratory Project Identification Number 414-139.

This study, under the direction of Study Director Zheng Chen, was conducted against the Bovine viral diarrhea virus (strain not specified; originally obtained from American BioResearch Laboratories; propagated in-house) using Madin Darby bovine kidney cells (MDBK cells; ATCC CCL-22) as the host system. The study protocol followed a MicroBioTest Protocol titled "Confirmatory Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for human Hepatitis C virus)," dated June 9, 2004 (copy provided). One lot (Lot No. 19125-1-2) of the product, Aseptrol S-Tab 10, was tested. A 199ppm use solution was prepared by adding two tablets of the product to a foil-covered flask containing 1200 ml of 400ppm AOAC synthetic hard water (titrated at 406ppm). The flask was allowed to react in the dark for 10 minutes, and was then swirled to dissolve any undissolved solids. The stock virus cultures contained at least a 5% organic soil load. Two glass carriers for the single product lot were tested against the target virus. Films of virus were made by spreading 0.2 ml of stock virus on the bottoms of separate sterile, glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. The use solution was sprayed onto the virus films from a distance of 6-8 inches until the surface was totally wet. The virus films were allowed to remain wet for 10 minutes at 22°C. After the contact period, 2.0 ml of horse serum containing 0.3% sodium thiosulfate was added to neutralize. The neutralized mixture was scraped from the surface of each dish with a cell scraper. Each sample was loaded onto pre-spun Sephacryl column. Following passage through the column, the eluate was removed. Ten-fold serial dilutions were prepared, using Minimum Essential Medium Eagle's containing 5% horse serum. MDBK cells were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at 37±2°C in 5±1% CO_2 for 3-5 days. The plates were assayed by a direct immunofluorescence assay. The $\log_{10}$ reduction in infectivity was determined using the Most Probable Number (MPN) method. Controls included those for cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed by titration.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Section E of the "Miscellaneous Information" section of the laboratory report (see page 28) states to "See attachments." The laboratory report does not have any attachments.

8. MRID 466417-08 "AOAC Use-Dilution Test Supplemental," Test Organism: *Escherichia coli* O157:H7 (ATCC 35150) for Aseptrol S-Tab 10, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – August 31, 2004. Laboratory Project Identification Number 414-137.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two lots (Lot Nos. 19125-1-1 and 19125-1-2) of the product, Aseptrol S-Tab 10, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. A 197ppm use solution (for Lot No. 19125-1-1) and a 222ppm use solution (for Lot No. 19125-1-2) were prepared by adding two 1.5 gram tablets of the product to a flask containing 1200 ml of 400ppm AOAC synthetic hard water (titration results not provided). The flask was covered with foil, and the use solution was allowed to react in the dark for 10 minutes. The flask was then swirled to dissolve any undissolved solids. Testing was not conducted in the presence of an organic soil load. Ten (10) stainless steel penicylinder carriers were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per 20.0 ml broth. The carriers were dried for 20-40 minutes at 37.2°C. Each carrier was exposed to 10 ml of the use

solution for 10 minutes at 20.2°C. After exposure, the carriers were transferred to tubes of Letheen Broth containing 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48.2 hours at 37.2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for sterility, viability, neutralizer effectiveness, bacteriostasis, dried carrier count, and confirmation of the challenge microorganism. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed using a HACH digital titrator kit.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Section E of the "Miscellaneous Information" section of the laboratory report (see page 23) states to "See attachments." The laboratory report does not have any attachments.

9. MRID 466417-09 "AOAC Use-Dilution Method, Test Organisms: *Staphylococcus aureus* Methicillin Resistant (ATCC 33592) and *Enterococcus faecalis* Vancomycin Resistant (ATCC 51575)" for Aseptrol S-Tab 10, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – July 30, 2004. Project Number A02258.

This study was conducted against *Staphylococcus aureus* - MRSA (ATCC 33592) and *Enterococcus faecalis* VRE (ATCC 51575). Two lots (Lot Nos. 19125-1-1 and 19125-1-2) of the product, Aseptrol S-Tab 10, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. A 213ppm use solution (for Lot No. 19125-1-1) and a 201ppm use solution (for Lot No. 19125-1-2) were prepared by adding three 1.5-gram tablets of the product to a container containing 1892.5 ml of 400ppm AOAC synthetic hard water (titrated at 402ppm). The use solution was allowed to stand in the dark for 15 minutes, and was then stirred until all of the solids were dissolved. Testing was not conducted in the presence of an organic soil load. Ten (10) stainless steel penicylinder carriers were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 1 carrier per 1.0 ml broth. The carriers were dried for 40 minutes at 35-37°C at a relative humidity of 52.6%. Each carrier was exposed to 10 ml of the use solution for 5 minutes at 20.1°C. After exposure, the carriers were transferred to 10 ml of Letheen Broth containing 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48.4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The equivalent chlorine dioxide concentration of the prepared use solution was confirmed by titration.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Antibiotic resistance of *Enterococcus faecalis* VRE (ATCC 41475) and *Staphylococcus aureus* - MRSA (ATCC 33592) were verified on representative cultures. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zones of inhibition confirmed antibiotic resistance of *Enterococcus faecalis* VRE to vancomycin and *Staphylococcus aureus* – MRSA to oxacillin. See Attachment I of the laboratory report.

V. RESULTS

MRID Number	Organism	Carriers	No. Exhibiting Growth/ Total No. Tested Lot No. 0407 22	Carrier Population (CFU/carrier)
466417-01	Bacillus subtilis	Sutures	1°=0/30 2°=0/30	4.9×10^5
		Penicylinders	1°=0/30 2°=0/30	3.1×10^5
	Clostridium sporogenes	Sutures	1°=0/30 2°=0/30	6.4×10^5
		Penicylinders	1°=0/30 2°=0/30	1.16×10^5

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Dried Carrier Count (CFU/carrier)
		Lot No. 19125-1-1	Lot No. 19125-1-2	
466417-08	Escherichia coli *O157:H7*	0/10	0/10	5.8×10^4
466417-09	Staphylococcus aureus - *MRSA*	0/10	0/10	1.01×10^7
466417-09	**Enterococcus faecalis *VRE***	0/10	0/10	2.8×10^6

MRID Number	Organism	Results			Dried Virus or Plate Recovery Control
			Lot No. 0407 22		
466417-02	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	5.05894 and 4.37983 log_{10}MPN/ml
		log_{10}MPN/ml	0.0	---	
			Lot No. 19125-1-1	Lot No. 19125-1-2	
466417-03	Newcastle disease virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$ $TCID_{50}$/0.1 ml
		$TCID_{50}$/0.1 ml	$10^{0.5}$	$10^{0.5}$	
466417-04	Duck Hepatitis B virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	4.7936 log_{10}MPN/ml
		log_{10}MPN/ml	0.0	0.0	
466417-05	Duck Hepatitis B virus	10^{-2} to 10^{-7} dilutions	---	Complete inactivation	4.7936 log_{10}MPN/ml
		log_{10}MPN/ml	---	0.0	
466417-06	Bovine viral diarrhea virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	6.0589 log_{10}MPN/ml
		log_{10}MPN/ml	0.0	0.0	
466417-07	Bovine viral diarrhea virus	10^{-2} to 10^{-7} dilutions	---	Complete inactivation	6.3798 log_{10}MPN/ml
		log_{10}MPN/ml	---	0.0	

VI CONCLUSIONS

A. Conclusions Regarding Use of the Product as a Sterilant

1. The submitted confirmatory efficacy data **support** the use of a 1000ppm solution of the product, Aseptrol S-Tab 10, as a sterilant against the following microorganisms in the presence of 400ppm hard water for a contact time of 1 hour:

Bacillus subtilis	MRID No. 466417-01
Clostridium sporogenes	MRID No. 466417-01

[The laboratory report assigned MRID No. 459160-01 (previously reviewed as part of the data package assigned D289783; E169) contains favorable efficacy results from initial/basic testing of three lots of the product according to DIS/TSS-9 standards.] Killing was observed in the subcultures of the required number of carriers (i.e., 30 carriers/2 types of surfaces) tested against the required number of product lots (i.e., 1). Carrier population counts were at least 10^4. Neutralization confirmation testing showed positive growth of the microorganisms. The viability controls were positive for growth. Purity controls were reported as pure. The sterility controls did not show growth. Test spores showed resistance to acid for $\geq$ 2 minutes.

B. Conclusions Regarding Use of the Product as a Disinfectant

1. The submitted efficacy data **support** the use of 200ppm solution of the product, Aseptrol S-Tab 10, as a disinfectant against the following microorganisms in the presence of 400ppm hard water on hard, non-porous surfaces at the contact times listed:

Staphylococcus aureus - MRSA	5 minutes	MRID No. 466417-09
Enterococcus faecalis VRE	5 minutes	MRID No. 466417-09
Escherichia coli O157:H7	10 minutes	MRID No. 466417-08

Killing was observed in the subcultures of the required number of carriers (i.e., 10) tested against the required number of product lots (i.e., 2). Carrier population counts were at least 10^4. Neutralization confirmation/neutralizer effectiveness testing showed positive growth of the microorganisms. The viability controls were positive for growth. When reported, purity controls were reported as pure. The sterility controls did not show growth.

2. The submitted efficacy data **support** the use of a 200ppm solution of the product, Aseptrol S-Tab 10, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400ppm hard water and a 5% organic soil load for a contact time of 10 minutes:

Feline calicivirus (surrogate for Norwalk virus) MRID No. 466417-02

Duck Hepatitis B virus (surrogate for Human Hepatitis B virus) MRID Nos. 466417-04 and -05

Bovine viral diarrhea virus (surrogate for Human Hepatitis C virus) MRID Nos. 466417-06 and -07

Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested. Recoverable virus titers of at least 10^4 were achieved. Initial and confirmatory studies against Duck Hepatitis B virus and Bovine viral diarrhea virus were performed at the same laboratory but under different study directors. Confirmatory studies used one lot of product, not the standard two.

3. The submitted efficacy data (MRID No. 466417-03) **support** the use of a 200ppm solution of the product, Aseptrol S-Tab 10, as a disinfectant with virucidal activity against the Newcastle disease virus on hard, non-porous surfaces in the presence of 400ppm hard water for a contact time of 5 minutes. A recoverable virus titer of at least 10^4 was achieved. Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

VII RECOMMENDATIONS

A. Recommendations Regarding Use of the Product as a Sterilant

1. The proposed label claims that a 1000ppm use solution of the product, Aseptrol S10-Tab, may be used for surface sterilization in areas where sterilization is required, such as those found in Level 3 and 4 Bio-safety Level facilities. Basic and confirmatory data provided by the applicant support this claim. The applicant must revised the proposed label as follows:

- The proposed label states that target surfaces must be thoroughly soaked/**wetted** or immersed for a contact time of 1 hour. Because it may be impossible to ensure that a soaked/wetted surface would remain exposed to the product for **1 hour**, the applicant should consider removing references to **wetting** surfaces.

- The proposed label does not list a pre-cleaning step in the Surface Sterilization directions. **In accordance with Section 101-2 of Subdivision H, labels for sterilants must include directions for pre-cleaning of the surfaces to be sterilized**. Pre-cleaning instructions must indicate to the user that pre-cleaned surfaces may be allowed to air dry or may be towel dried. Surfaces cannot be dried using dry heat.

- The proposed label does not specify a reaction/activation time in the preparation directions for 1000ppm solutions. The applicant must add the following sentence: "**Wait 15 minutes for the 6-gram tab to completely dissolve.**"

- Because there are many types of plastics used by the health-care industry, the label must indicate the type(s) of plastics with which the product is compatible (e.g., polystyrene, polypropylene, and polyvinyl chlorides) or those plastics with which the product is incompatible.

- A statement similar to the following should immediately follow the directions for use of the product as a sterilant: "When sterilizing complex medical instruments such as [Identify the type of medical/dental instruments intended for treatment, in addition to any description of surface composition]: Fully disassemble the instrument according to manufacturer's instructions; clean and rinse the lumens of all hollow instruments (making sure the instrument is completely free of all soap before immersion in the product use-solution); sterilize the instrument according to product label directions (all lumens must

be completely filled with the product use-solution; avoid entrapment of air bubbles) and completely reassemble under aseptic conditions".

B. Recommendations Regarding Use of the Product as a Disinfectant

1. The previously submitted efficacy data (MRID No. 462665-01) **support** the proposed label claims that a 100ppm solution of the product, Aseptrol S10-Tab, is an effective disinfectant against the Foot and Mouth Disease virus after 30 minutes of contact. **The applicant must specify 30 minutes contact time for Foot and Mouth Disease virus on page 2 of the proposed label, under "Laboratory animal facilities, animal rearing..." paragraph.**

2. The proposed label claims that a 200ppm solution of the product, Aseptrol S10-Tab, is an effective disinfectant against the following microorganisms at the contact times listed:

Hepatitis B virus	10 minutes
Hepatitis C virus	10 minutes
Newcastle Disease virus	10 minutes
Norwalk virus	10 minutes
Escherichia coli O157:H7	10 minutes
Staphylococcus aureus - MRSA	5 minutes
Enterococcus faecalis VRE	5 minutes

Data provided by the applicant **support** these claims.

3. The proposed label now claims that a 200ppm solution of the product, Aseptrol S10-Tab, is an effective disinfectant against the following microorganisms at a 10-minute contact time:

Human coronavirus
Bordetella bronchiseptica
Corynebacterium bovis
Helicobacter pylori

Data previously provided by the applicant (and reviewed as part of the data package assigned D304976; E328) **support** these claims.

4. The proposed label now claims that a 50ppm solution of the product, Aseptrol S10-Tab, may be used to sanitize hard, non-porous, food contact surfaces at a contact time of 60 seconds. Data previously provided by the applicant (and reviewed as part of the data packages assigned D304976; E328 and D289783; E169) **support** this claim.